reporter cell, leading to expression of the luciferase reporter gene and dose-dependent luciferase signal, as shown in FIG. **1**C. Two BCMA×CD3 antibodies were tested, with the dotted lines indicating the concentration used in subsequent assays.

[0085] These results show that the cell-based assays of the invention provide a robust dose response curve and predictably respond to positive and negative controls.

Example 2. Detection of NAbs Against a Therapeutic Protein Using a Cell-Based NAb Assay

[0086] This example shows further proof of concept of the experimental design of the NAb assay of the present invention. In a cell-based NAb assay, NAbs against a therapeutic protein inhibit binding of the therapeutic protein to its target and/or reporter cells, and thereby eliminate reporter signal. The reduction of reporter signal or activity in the NAb assay is a measure of the presence of NAbs in the sample.

[0087] For example, FIG. **2**A illustrates the action of NAbs against a bispecific CD20×CD3 drug antibody, wherein binding of NAbs against the anti-CD20 arm or anti-CD3 arm of the bispecific antibody interrupts binding to CD20 or CD3 respectively, eliminating luciferase activity. To further validate this cell-based NAb assay, surrogate NAbs were added to the NAb assay, targeting either the anti-CD20 arm or anti-CD3 arm of the bispecific CD20×CD3 drug antibody. Addition of NAbs caused a decrease in luciferase activity in a dose-dependent manner, as shown in FIG. **2**B.

[0088] The effectiveness of this cell-based NAb assay was further validated for use with two bispecific BCMA×CD3 drug antibodies. Surrogate NAbs were added to the NAb assay, targeting either the anti-BCMA arm or anti-CD3 arm of the two bispecific BCMA×CD3 drug antibodies. Addition of NAbs caused a decrease in luciferase activity in a dose-dependent manner, as shown in FIGS. **2**C, **2**D, **2**F and **2**G. Addition of isotype controls had no effect on luciferase activity, as shown in FIGS. **2**E and **2**H.

[0089] These results show that the assay of the present invention reliably measures the presence of neutralizing antibodies against a therapeutic protein in a dose-dependent manner.

Example 3. Cell-Based NAb Assay Interference by a Competing Drug

[0090] NAb assays may be susceptible to false positive or false negative results due to interference from matrix components. One potential source of interference is a second drug that competitively binds to the target of the therapeutic protein being tested. As a proof of concept of this type of interference, NAb assays for a bispecific CD20×CD3 drug antibody were conducted with the addition of competing antibodies against either CD20 or CD3, as shown in FIG. **3**A and FIG. **3**B. The addition of a competing drug caused a dose-dependent reduction in luciferase activity, mimicking the reduction in luciferase activity caused by surrogate NAbs and therefore producing a false positive result.

[0091] Interference from competing drugs was also seen in NAb assays for two bispecific BCMA×CD3 drug antibodies. The addition of bivalent parental antibodies against BCMA or CD3 caused a decrease in luciferase signal in both assays, as shown in FIGS. **3**C and **3**E. The addition of various clinical candidate antibodies against BCMA also caused a decrease in luciferase signal in both assays, as shown in FIGS. **3**D and **3**F.

[0092] These results demonstrate proof of concept that the presence of a competing second drug can produce a false positive result in a cell-based NAb assay.

Example 4. Addition of Human Serum to a Cell-Based NAb Assay

[0093] As discussed above, NAb assays may be susceptible to interference from matrix components. To test the resilience of the NAb assay of the invention to potential interference, the NAb assay for a bispecific CD20×CD3 drug antibody was conducted with the addition of drug-naïve human serum as shown in FIG. **4**A. Luciferase activity was unaffected by the addition of human serum, demonstrating the resilience of the NAb assay of the invention to interference from human serum components and therefore suitability for clinical application.

[0094] FIG. **4**B demonstrates a simple representation of "NAb assay signal". The relative presence of NAbs in a sample is quantitated by dividing luciferase activity induced with a drug control over luciferase activity induced in an experimental sample. Luciferase activity is reduced in a dose-dependent manner in the presence of NAbs, leading to a higher NAb assay signal.

Example 5. Cell-Based NAb Assay Interference in Clinical Samples

[0095] The NAb assay of the present invention was used to test 60 drug-naïve human samples from a clinical trial for the presence of NAbs against a bispecific CD20×CD3 drug antibody, as shown in FIG. **5**. Although the tested patients had not been exposed to the drug antibody, many samples showed a false positive result for NAbs.

[0096] As discussed in Example 3, one possible source of a false positive signal in a NAb assay is a competing second drug. Many patients in this clinical trial had a history of prior anti-CD20 therapy. In order to assess whether a competing anti-CD20 drug may be responsible for the false positive results of the NAb assay, a subset of 17 human samples were tested for the presence of rituximab, an anti-CD20 antibody, using a commercially available ELISA. The presence of rituximab correlated with false positive NAb assay signal, as shown in FIG. **6**.

[0097] These results demonstrate that interference from a residual competing drug may result in false positive results in a NAb assay in a clinical application, and must be addressed in order to accurately detect NAbs against a therapeutic protein.

Example 6. Mitigation of Cell-Based NAb Assay Interference by a Competing Drug

[0098] As described above, the presence of a competing drug may interfere with the binding of a therapeutic protein to its target in a NAb assay, resulting in reduction of reporter activity and a false positive NAb assay signal. This is illustrated in FIG. **7**A, using the example of a bispecific CD20×CD3 drug antibody as the therapeutic protein and rituximab, an anti-CD20 antibody, as the competing drug. In order to accurately detect NAbs against a therapeutic protein in the presence of a competing drug, binding of the competing drug to the mutual target must be mitigated. This is